DECIDUOMAL RESPONSE TO UTERINE TRAUMA FOLLOWING CERVICAL STIMULATION IN IMMATURE RATS WITH LUTEINIZED OVARIES*

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Weanling rats with ovaries heavily luteinized by injections of 40 I.U. PMS and 20 I.U. HCG fail to form deciduomata in response to uterine traumatization, although the corpora lutea induced in their ovaries are capable of secreting some progestins as evidenced by mucification of the vaginal epithelium following administration of estrogen. Daily injections of prolactin, alone or in combination with LH, are totally ineffective in rendering such rats responsive to uterine traumatization (Armstrong and Greep, 1965; Takewaki, 1969a).

If the ovaries are mildly luteinzed by injections of 5 I.U. PMS and 2 I.U. HCG in similar immature rats, subsequent estrogen injections result in vaginal cornification showing that progestins are no longer secreted in amounts sufficient to synergize with the exogenous estrogen to cause vaginal mucification. However, in a majority of animals so primed but not given estrogen, daily injections of prolactin are effective in enabling the formation of deciduomata following uterine traumatization (Takewaki, 1969a).

The writer (1969b) has shown that, in immature rats ovariectomized after having been primed with large doses of PMS and HCG, daily injections of a combination of estradiol and progesterone which can promote optimal uterine sensitivity as well as optimal maintenance of deciduomal growth in ovariectomized adult rats fail to elicit deciduomal response to uterine trauma. Since deciduomata are readily obtained by similar procedures in immature rats ovariectomized prior to the PMS-HCG priming, it is evident that treatment with large doses of PMS and HCG renders the uteri refractory to trauma through the intermediary of the stimulated ovaries. Weanling rats ovariectomized after priming with small doses of PMS and HCG are capable of forming well developed deciduomata in reaction to uterine traumatization,

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if they are placed on a similar daily injection regimen of progesterone and estradiol.

The experiments dealt with in this paper were undertaken to study the effects of mechanical cervical stimulation on the uterine sensitivity to trauma in immature rats pretreated with PMS and HCG. The results again showed that the cervical stimulation was effective in enabling the development of deciduomata following uterine traumatization in animals with ovaries mildly luteinized by injections of small doses of PMS and HCG but not in those bearing ovaries strongly luteinized by large doses of the gonadotropins.

Materials and Methods

All animals used were female rats of the T strain raised in an artificially illuminated (14 hrs light-10 hrs dark), temperature-controlled room. At 21 days of age, rats were given a single subcutaneous injection of PMS (Primantron, Schering A. G., Berlin), followed 2.5 days later by a single injection of HCG (Primogonyl, Schering A. G., Berlin). All rats were weaned on the day of PMS injection.

Experiments were carried out on 2 groups of animals: one receiving 5 I.U. PMS and 2 I.U. HCG and the other, 40 I.U. PMS and 20 I.U. HCG. The doses were dissolved in 0.1 ml of a 0.9% NaCl solution. Immature rats primed with the gonadotropins usually had the vaginae open on the day following HCG injection, vaginal cornification taking place on that or the next day.

The region of the cervix uteri and posterior fornix of the vagina was rapidly tapped 50-60 times with a glass rod, 2 mm in diameter, on the day of vaginal estrus. De Feo (1963) reported that this technique of cervical stimulation on the day of estrus was highly effective in activating newly formed corpora lutea and inducing pseudopregnancy in adult rats.

Four days after cervical stimulation, uterine traumatization was performed in each animal under ether anesthesia. The antimesometrial side of the endometrium was scratched along the entire length of the right uterine horn by a needle with a bent point inserted into its lumen via a small incision made near the cervical end of the horn (Takewaki, 1969a).

At autopsy 4 days later, traumatized uterine horns were examined for gross evidence of deciduomata. In animals given small doses of gonadotropins, corpora lutea in each ovary were counted. Ovaries,

and traumatized and intact uterine horns were weighed. Since animals primed with large doses of PMS and HCG invariably failed to form deciduomata, the combined weights of both horns were recorded for them. Ovaries, uteri and vaginae were fixed in Bouin's solution. Sections cut in paraffin were stained with Delafield's hematoxylin and eosin.

All the experiments were carried out at the Zoological Institute, Faculty of Science, University of Tokyo.

Results

Failure of uteri to respond to traumatization in rats primed with 40 I.U. PMS and 20 I.U. HCG.

In a group of 13 rats, neither gross nor histological examinations of traumatized uterine horns 4 days after traumatization revealed any evidence of formation of deciduomata. Ovaries were heavily luteinized, averaging 104.4 ± 7.67 mg in weight. Uteri weighed 82.3 ± 5.06 mg on the average. The rats showed leucocytic vaginal smears continuously from the day following cervical stimulation until sacrifice. At autopsy the vaginal epithelium was mucified.

Formation of deciduomata in response to uterine traumatization in rats primed with 5 I.U. PMS and 2 I.U. HCG

At autopsy 4 days after uterine traumatization, 8 of 9 rats showed the deciduomal response involving the entire endometrium from the cervix to the uterotubal junction of the traumatized horns. In 1 of the 8 animals a deciduomal swelling was also found in the left non-traumatized horn. The horns bearing deciduomata of the 8 rats weighed 234.9 ± 29.04 mg on the average, while the contralateral horns without deciduomata of the 7 animals, 37.0 ± 1.61 mg. Ovaries of the 8 rats contained 6–15 corpora lutea per animal, weighing 32.6 ± 1.79 mg on the average.

Diestrous type vaginal smears continued from the day following cervical stimulation until sacrifice. Copious mucus often appeared in the smears. Histological studies revealed that the vaginal epithelium was mucified in all the animals.

Discussion

Following mechanical stimulation of the cervical region, a majority of immature rats with ovaries mildly luteinized by injections of 5 I.U. PMS and 2 I.U. HCG developed deciduomata in response to uterine

traumatization. If the cervical region was not stimulated, similarly primed immature rats were totally unresponsive to uterine trauma, showing that the induced corpora lutea were incapable of secreting progesterone in amounts sufficient to render their uteri reactive to trauma (Takewaki, 1969a).

Since evidence for the luteotropic activity of prolactin appears to be unequivocal in the rat (Meites, 1966), it is probable that cervical stimulation by the method highly effective in activating newly formed corpora lutea in adult rats (De Feo, 1963; Carlson and De Feo, 1965) was likewise effective in initiating secretion of prolactin from the anterior hypophysis in immature rats. Herlyn, Geller, v. Berswordt-Wallrabe and v. Berswordt-Wallrabe (1965) have rerorted that cervical stimulation at full estrus in adult rats is followed by a decrease in hypophysial content of prolactin within 30 minutes indicating an increased release of the hormone from the hypophysis.

By contrast, immature rats with ovaries heavily luteinized by injections of 40 I.U. PMS and 20 I.U. HCG invariably failed to develop deciduomata in response to uterine traumatization following cervical stimulation.

It has been shown previously (Takewaki, 1969a; Takewaki and Machida, 1970) that induced corpora lutea in immature rats are capable of producing progestins in considerable amounts only when they are present in excessive numbers. However, it is inconceivable that progestins produced by heavily luteinized ovaries would interfere with the enhancement of prolactin secretion from the anterior hopophysis following cervical stimulation, in view of the finding of Rothchild and Schwartz (1965) that progesterone is effective in maintaining the hypophysial secretion of prolactin.

It is also improbable that corpora lutea in heavily luteinized ovaries of immature rats are refractory to prolactin. Electron-microscopical studies by Rennels (1966) revealed that luteal cells in ovaries of hypophysectomized immature rats bearing hypophysial transplants under the renal capsule appeared equally active whether the ovaries were heavily luteinized by injections of 50 I.U. PMS and 25 I.U. HCG or they were mildly luteinized by priming with 4 I.U. PMS alone. Luteal cells in ovaries of immature rats similarly primed and hypophysectomized but not given hypophysial grafts were shrunken and inactive.

Wiest, Kidwell and Balogh (1968) and Hashimoto and Wiest (1969a,

b) postulated that LH stimulates the conversion of progesterone to 20α-hydroxypregn-4-en-3-one (20α-OH-P) by augmenting the activity of 20α-hydroxysteroid dehydrogenase (20α-OH-SDH), while prolactin sustains the levels of progesterone by arresting or decreasing the activity of the enzyme. On the other hand, diurnal fluctuation of ascorbic acid content in ovaries of immature rats heavily luteinized by injections of PMS and HCG probably associated with periodic release of LH from the anterior hypophysis has been pointed out by Lawton and Schwartz (1965) and de Groot (1967). Accordingly, the failure of rats so primed to develop deciduomata in response to uterine trauma may be accounted for by the luteolytic effects of the LH. However, whether or not the pattern of LH secretion would be affected by cervical stimulation in such animals has not been worked out as yet.

In adult rats made pseudopregnant by mechanical stimulation of the cervix uteri, Schwartz and Rothchild (1964) reported a fairly progressive rise in LH concentration in the anterior hypophysis from the first day of diestrus through the 11th day. It seems likely that this inhibition of LH release from the anterior hypophysis is concerned with maintaining the luteal function during pseudopregnancy. On the 12th day a significant drop in the hypophysial LH concentration took place, undoubtedly reflecting the discharge of LH associated with ovulation. Yet the LH contents in the anterior hypophysis of immature pseudopregnant rats with ovaries mildly luteinized by small doses of PMS and HCG remain to be studied.

The most likely explanation now available for the failure of deciduomal response in immature rats receiving cervical stimulation after priming with large doses of PMS and HCG appears to be that the uteri of such rats are totally unresponsive to traumatization even if they are subjected to the influence of progesterone and estradiol in the optimal combination for inducing deciduomal development (Takewaki, 1969b). Any interpretation which fails to consider the uterine sensitivity to trauma in immature rats primed with PMS and HCG as a complicating factor must be accepted with considerable reservation.

Summary

Weanling rats primed with 40 I.U. PMS and 20 I.U. HCG invariably failed to form deciduomata in response to uterine trauma applied after mechanical stimulation of the uterine cervix, while a majority of

those injected with 5 I.U. PMS and 2 I.U. HCG developed deciduomata under similar circumstances. The results were discussed in relation to a) the secretion of prolactin from the anterior hypophysis, b) the reactivity of the induced corpora lutea to prolactin, c) the luteolytic effect of LH and b) the sensitivity of the uteri to trauma.

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